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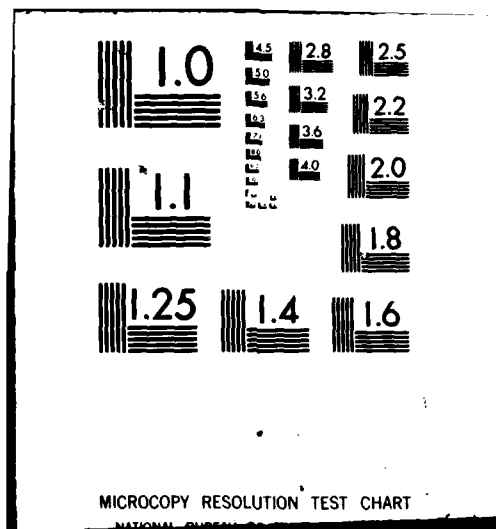
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DOXYCYCLINE PROPHYLAXIS OF SCRUB TYPHUS

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Scrub typhus is a febrile illness caused by infection with Rickettsia tsutsugamushi, the vectors of which are larval stages of trombiculid mites, frequently referred to as chiggers.

Although the disease was originally reported only from restricted areas in Japan, it is now known to have a widespread distribution within a large triangular area bounded by Siberia in the north, Australia in the south, and Pakistan in the west. The distribution of the disease parallels that of known vector mites.

Scrub typhus gained military importance among both Allied and Japanese forces during World War II. Records are incomplete on casualties suffered from the disease by Japanese forces, but estimates approaching 30,000 have been made (1). Among Allied forces, available statistics indicate that approximately 18,000 casualties occurred (2). Of 11,000 cases for which adequate data are available, there were nearly 650 deaths. In American troops, case fatality rates varied in different epidemics between less than one percent and thirty-five percent. Scrub typhus seriously jeopardized the operational efficiency of military units in which the sudden outbreaks occurred. In Vietnam, scrub typhus was a leading cause of fever of unknown origin in US soldiers (3,4).

The lack of an effective vaccine for scrub typhus has caused other methods of prevention to be considered. One method, dating from the 1940's, is chemoprophylaxis with antibiotics. Once weekly 4g doses of chloramphenicol were shown to provide successful scrub typhus prophylaxis if taken for a minimum of four weeks after exposure (5). Recognition of the risk of dose-related and idiosyncratic bone marrow toxicity, however, now precludes the prophylactic use of this antibiotic (6,7).

Tetracyclines are currently the drugs of choice for therapy of scrub typhus (8). In this group, doxycycline and minocycline have the most

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group received their placebo capsules under the same circumstances. No other medications were allowed to be taken during the study except that dextropropoxyphene napsylate 100 mg (Eli Lilly) was available as an analgesic. Because this drug does not have an antipyretic effect (21), there was no masking of febrile responses in the volunteers.

Surveillance and criteria for diagnosis. All subjects were examined daily, and oral temperatures were recorded twice daily. Upon completion of the seven-week course of prophylaxis, the remaining healthy volunteers were kept under clinical surveillance for a further four weeks. During the prophylaxis period, venous blood was drawn every three days for standard hematological and biochemical tests, indirect FA, and rickettsial isolation. Subsequently, blood was drawn every week for two months. Indirect FA was measured against each of eight prototype strains of R. tsutsugamushi: Karp, Kato, Gilliam, TA678, TA686, TA716, TA763 and TH1817. Titers were expressed as the reciprocal of the highest positive serum dilution. A change in the indirect FA titer from zero (<25) to 50 or a four-fold rise between initial and subsequent sera was considered significant.

For rickettsial isolation, 0.2 ml of each blood sample was inoculated ip into three adult white mice. Twenty-eight days later, surviving mice were challenged ip with 10^3 mouse LD₅₀ of the Karp strain of R. tsutsugamushi. Death after the first inoculation or survival after the challenge indicated the presence of R. tsutsugamushi in the original blood specimen (22). Further aliquots of each venous blood sample were stored at -70C. Subsequently, 0.2 ml of each blood sample was inoculated ip into five glycogen-treated mice. When these animals became sick or on day 14, peritoneal cells were harvested and examined for R. tsutsugamushi by the direct FA (23) test.

A clinical diagnosis of scrub typhus was based on either (1) co-existence of two or more of the cardinal signs - eschar, generalized lymphadenopathy, hepatomegaly and/or splenomegaly, and rash - or (2) fever greater than 37.6C for more than 48 hours.

Subjects considered to have scrub typhus on clinical grounds were treated on the third day of their illness with a single 200 mg oral dose of doxycycline. Recovery from scrub typhus was defined as absence of symptoms and a 24-hour period with temperature less than 37.2C. Relapse was defined as the presence of fever greater than 37.6C for 24 hours or the recurrence of fever in any two consecutive 24 hour periods after apparent recovery from scrub typhus. Therapy for relapse was tetracycline hydrochloride 500 mg sixth hourly for seven days.

Statistics. Prior to the study, an estimate of the required group sizes was made, using various infection rates for the placebo and drug group and a significance level of $p = 0.01$. For example, if the disease rates in placebo and drug groups were 0.8 and 0.2 respectively, eight subjects would be needed in each group for a significance level of $p = 0.01$. We chose 20 volunteers as a minimum number to allow for the possibility that some volunteers might withdraw from the study. Our results were analyzed in a two-tailed test of significance by Fisher's exact probability test.

Results

Chigger attachment. The day after the first dose of prophylaxis, a small plastic capsule (24) was glued to the medial aspect of the thigh of each volunteer. The next morning, adherence of the capsule was checked and ten chiggers were placed in each capsule. The chiggers were observed under a dissecting microscope for 15 minutes, by which time some had begun to attach in about half the volunteers. A dampened cotton swab was placed in the capsule to prevent dessication of the chiggers, and the cover of the capsule was taped closed. For those volunteers with slow attachment, the chiggers were replaced with a fresh batch and observed. In this way, 16 volunteers had chiggers attached on the third day after prophylaxis was started. Attachment and condition of the chiggers were rechecked later in the day. The four volunteers (two from each treatment group) with no chigger attachment had fresh chiggers inserted in the late afternoon and left overnight. The following day all of these volunteers had some chiggers attached.

Between two and 13 (mean 8.5) chiggers attached to each volunteer and between two and 13 (mean 7.1) engorged chiggers were recovered 48 hours after attachment. Direct FA staining of these engorged chiggers (25) showed all to be infected with R. tsutsugamushi.

Prophylaxis period. No significant adverse effects from doxycycline were reported. Mild epigastric discomfort was noted after one dose by two subjects; one other dose was vomited by one of these subjects and was repeated.

Starting between the third and ninth days post chigger attachment (PCA), all subjects, except three receiving doxycycline, developed tender inguinal lymphadenopathy adjacent to the site of chigger attachment. The three remaining subjects had similar findings during the succeeding eight days. By day 7 PCA, all the placebo group and two of the drug group had a small, indurated, erythematous nodule at this site. In none of the drug group did this progress, whereas in all but one of the placebo group a classical eschar developed. The one exception was an American volunteer,

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who had no past history of scrub typhus and no pre-existing scrub typhus antibody, but had had several years of occupational (laboratory) exposure to scrub typhus rickettsiae.

Between days 7-11 PCA, nine of the placebo group and one of the drug group were hospitalized with presumed scrub typhus (Table 1). The clinical diagnosis was subsequently confirmed by isolation of rickettsiae from blood with or without a four-fold rise in indirect FA titer.

TABLE 1. Scrub typhus in subjects receiving placebo or doxycycline prophylaxis*

Group	Number of subjects with	
	Scrub typhus	No disease
Placebo	9	1
Doxycycline	1	9

* $p = 0.001$ (two-tailed test of significance by Fisher's exact probability test).

The one placebo subject not diagnosed as having scrub typhus had pre-existing antibody. He never had fever but formed an eschar and in the third week PCA, as the eschar healed, developed generalized lymphadenopathy. He was not treated and recovered spontaneously.

The nine subjects in the doxycycline group who did not become ill reported some symptoms in the period from days 9-24 PCA. Malaise was the most common complaint, with abdominal pain and arthralgia occurring less frequently. In several volunteers there was a waxing and waning both of the minimal induration at the chigger attachment site and of the adjacent lymphadenopathy. Three subjects had intermittent fever $\leq 37.3^{\circ}\text{C}$. Symptoms were maximal at the time of the next dose and resolved within 24 hours of medication. After day 24 PCA all symptoms were absent. However, in the fourth week PCA, five of the nine had a mild generalized non-tender lymphadenopathy.

Post-prophylaxis period. Ten to 14 days after the last dose of doxycycline, eight of the nine remaining subjects reported or exhibited further abnormalities that lasted up to seven days. These included malaise (four cases), abdominal pain (two cases), arthralgia (one case),

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and lymphadenopathy (seven cases). Three subjects had low-grade fever ($<37.6^{\circ}\text{C}$) for one, two and four days. All these abnormalities were self-limiting. The volunteers were able to continue their normal duties except for one who was given sedentary work for one day.

Therapy. Subjects with scrub typhus were treated on the third day of disease, 48-64 hours after fulfilling the diagnostic criteria. Within 36 hours of receiving their single dose of doxycycline, all 10 patients with scrub typhus were afebrile and virtually symptom-free. However, five to ten days after therapy, all experienced a recurrence of one or more symptoms - headache, malaise, myalgia, arthralgia, photophobia. Six of the 10 were treated for relapse. A seventh patient had such severe constitutional symptoms, combined with tender hepatosplenomegaly and generalized lymphadenopathy, that he also was treated for a relapse. A week long course of tetracycline brought about rapid recovery in all seven subjects, and there were no further relapses. The three subjects with milder symptomatology, but no fever, recovered spontaneously.

Hematology. There was no characteristic alteration in total or differential leukocyte counts in the 10 subjects treated for scrub typhus. Platelet counts were $<200 \times 10^9/l$ in eight subjects during their acute illness, and in two of these the counts were $<150 \times 10^9/l$. There were no sequelae of the thrombocytopenias.

Biochemistry. One placebo subject had a mild elevation of aspartate aminotransferase (AST) during his acute scrub typhus infection. All other values of AST, lactic dehydrogenase, alkaline phosphatase, urea, and electrolytes were normal for all subjects in the study.

Evidence of infection. All subjects had rickettsiae isolated from peripheral blood at some stage during the study. In those developing scrub typhus, rickettsemia was demonstrated up to three days prior to the onset of fever and was frequently present three days after apparently successful therapy, when the subjects had neither fever nor symptoms. No rickettsiae were isolated from blood drawn at the time of relapse after single-dose therapy.

All placebo subjects and all but one doxycycline subject had at least four-fold rises in indirect FA titer to one or more strains of R. tsutsugamushi. The one exception was the sole failure of doxycycline prophylaxis; he had a low pre-existing indirect FA titer that remained unaltered despite clinical scrub typhus (with rickettsial isolation on four successive blood samples) and relapse.

Volunteers receiving doxycycline and remaining well developed significant antibody titers during the fourth week PCA. This

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corresponded to the time that most had a mild generalized lymphadenopathy. Antibody titers were declining in most subjects by the end of the seven week prophylaxis period. In the subsequent two months, however, three subjects had a significant rise in antibody titer and four other subjects had consistent two-fold rises in antibody.

Discussion

We conclude from our data that doxycycline in weekly 200 mg doses provides effective chemoprophylaxis of naturally transmitted scrub typhus if prophylaxis is started before exposure to infection and continued for six weeks after exposure ($p = 0.001$).

Laboratory-reared chiggers from our colony have previously been used to transmit scrub typhus to three volunteers (19). The isolation of *R. tsutsugamushi* from all 20 volunteers in this study further attests to the reliability of this method, which simulates a naturally acquired infection.

The question arises why weekly prophylaxis is effective. Chloramphenicol and the tetracyclines are bacteriostatic agents and their roles in the therapy of scrub typhus are attributed to suppression of rickettsial growth while the body develops an immune response (25). *Rickettsiae* have slower *in vitro* growth characteristics than most other bacteria, having a doubling time of approximately 10 hours (27). Appropriately timed prophylaxis can therefore allow sufficient increase in the antigenic load to induce immunity while preventing overt disease.

We have compared very closely the results of our study with those from previous scrub typhus chemoprophylaxis trials using chloramphenicol (5,28-30). Using an artificial method for inoculating rickettsiae, Smadel *et al* derived a prophylactic regimen (30) that provided protection without any symptoms either during or following the prophylaxis period. However, in all their studies employing natural means of infection and lasting longer than three weeks, they noted fever $>37.8^{\circ}\text{C}$ during the second and third weeks of prophylaxis around the time of the next dose of prophylaxis. The number of rickettsiae transmitted by each chigger in a naturally acquired infection is unknown but may well be greater than the dosage used in inoculation studies (29,30). The symptoms reported by our volunteers in the second and third weeks were mild by comparison, and fevers, all $\leq 37.3^{\circ}\text{C}$, were observed in only three subjects. Some immunity is presumably developing by the time these symptoms completely disappear because significant antibody levels were found in eight of our nine subjects at this time.

If we assume that after four half-lives neither drug is suppressive, then a once weekly dose of doxycycline ($t_{1/2}$ 15 hours) would be effective for 60 hours, with a therapeutic hiatus of 4.5 days. Equivalent therapy would be achieved with chloramphenicol ($t_{1/2}$ 2.5 hours) given every five days. Approximately this interval was used in a Gilliam strain inoculation study (29), and mid-prophylaxis fevers, but no late symptoms, were observed in subjects who received prophylaxis for at least 21 days. With a slightly shorter interval (29), complete protection with no symptoms was achieved following the inoculation of Karp strain.

Minor modifications to our regimen might achieve a similar result. For example, a six day interval and a slightly lower dose may permit optimal rickettsial multiplication to stimulate immunity while preventing clinical disease. Although doxycycline is moderately expensive, our prophylaxis regimen may have a role in preventing scrub typhus in personnel temporarily exposed to infection in high-risk areas. Furthermore, doxycycline may be appropriate for use in a combined chemoprophylaxis-living vaccine using several strains of *R. tsutsugamushi*. Long-lasting homologous immunity develops after infection suppressed with chloramphenicol (31), and we expect similar immunity after doxycycline prophylaxis. Also we have found recently that some strains which are avirulent for laboratory animals protected monkeys against a subsequent challenge with a virulent strain (unpublished results). This may simplify the choice of vaccine strains.

The demonstration of rickettsemia as early as three days before clinical disease and for as long as three days (five days in one case) after apparently successful therapy extends previous data (30,32). Only those who relapsed had rickettsemia demonstrated two or more days after initial therapy but we were surprised at our inability to isolate rickettsiae from blood collected at the time of relapse. The symptoms experienced by the majority of the doxycycline group during the post-prophylaxis period were probably due to the multiplication of rickettsiae that was insufficient to cause serious clinical disease. Although no rickettsemias were demonstrated at this time, three subjects had significant rises in antibody titer and four subjects had one dilution rises in titer after prophylaxis was stopped.

Our findings on eschar formation were different from previous reports of scrub typhus chemoprophylaxis. Although Alson *et al* (16) in a doxycycline field study did observe eschar in some prophylaxis subjects, these subjects were necessarily failures of prophylaxis by virtue of their study design. Smadel *et al* (29) found eschar in eight of 28 prophylaxis subjects who were inoculated with rickettsiae intradermally. However, in field studies, Smadel *et al* (5) did not observe classical eschar in any prophylaxis subject. Similarly, none of our prophylaxis subjects,

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not even the one failure, developed eschar.

All eight Asian subjects in our control, regardless of their pre-existing antibody titer, developed classical eschar. The incidence of eschar in both non-immune and immune Asian subjects has been reported to be very low (28,33). Our data do not support the hypothesis that partial immunity prevents eschar formation even though generalized disease might occur (28). The large number of chiggers attaching to some volunteers does not explain the high frequency of eschar in our control group. Even two chiggers were sufficient to cause eschar and disease in one Malaysian volunteer. Furthermore, our method of transmitting infection may not have been very different from the natural situation because chiggers in nature tend to cluster in one area on the body before attaching.

The poor results of early, single-dose doxycycline therapy for scrub typhus were not unexpected. Previous experience with chloramphenicol and terramycin showed a high incidence of relapse after short courses of treatment early in the disease (26). The originators of single-dose doxycycline therapy for scrub typhus (14) were unable to draw any conclusions on the merits of early treatment because their patients were treated on mean day 10 of disease. We believe that antibiotic treatment for scrub typhus should span at least seven days unless therapy is started late in the disease.

The findings of this study have provided the basis for a recommendation that military personnel deployed to a scrub typhus endemic area be given weekly doses of 200 mg doxycycline to protect them against the disease.

Acknowledgments

The study was approved by three human use committees at the Institute for Medical Research, Kuala Lumpur, Malaysia, at the Walter Reed Army Institute of Research, and the US Army Human Subjects Research Review Board, Washington, DC. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Reg 70-25 on Use of Volunteers in Research.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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